Gene expression profile and aortic vessel distensibility in voluntarily exercised spontaneously hypertensive rats: potential role of heat shock proteins

Ulrika Hägg, Maria E. Johansson, Julia Grönnos, Andrew S. Naylor, Ingibjörg H. Jonsdottir, Göran Bergström, Per-Arne Svensson, and Li-ming Gan

1Department of Physiology, Institute of Physiology and Pharmacology, Göteborg University; 2Department of Clinical Physiology, Cardiovascular Institute, Sahlgrenska University Hospital; 3Institute of Stress Medicine; and 4Department of Internal Medicine, Research Centre for Endocrinology and Metabolism, The Sahlgrenska Academy, Göteborg University, Göteborg, Sweden

Submitted 29 March 2005; accepted in final form 20 May 2005

Exercise is known to increase not only the production of free radical oxygen species (ROS) (7) but also the synthesis of superoxide dismutases (SODs) (15), a phenomenon also confirmed in our laboratory’s previous study (13). There is increasing evidence that oxidative stress plays a critical role in the pathogenesis of atherosclerosis (5). The cellular stress response to oxidative stress includes the heat shock proteins (HSP), a multigene family that range from 10 to 150 kDa in molecular size and show highly homologous sequences between different species. Among the HSP proteins, HSP60 has gained special interest in atherosclerosis. Serum-soluble HSP60 levels have been shown to correlate with carotid artery atherosclerosis in humans (28). However, possible effects of physical exercise on vascular HSP expression still remain unclear.

In the present study, we aim to further investigate effects of physical exercise on vascular mechanical properties and explore gene regulation patterns in response to chronic voluntary exercise.

MATERIALS AND METHODS

Animals

The experiments were performed on 18 female spontaneously hypertensive rats (SHR) (Taconic M & B Breeding & Research Centre, Bomholmgaard, Denmark). Animals arrived at the age of 8 wk and were acclimatized for 1 wk before onset of the experiment. All animals were housed at constant temperature (24°C) at a relative humidity of 50–60%. A 12-h dark-light cycle was maintained in the animal room. The rats had free access to standard pellet chow and tap water. Animals were weighted weekly. The experimental protocol was approved by the Regional Animal Ethic Committee, Göteborg University.

Computerized Wheel Cage Model for Voluntary Exercise

This model has previously been described in detail (36). In brief, rats were randomly divided into one exercising group (n = 9) and one control group (n = 9). All animals were allocated individually into separate cages of the same size (41 × 31 × 23 cm) for 5 wk. Rats assigned to the exercising group had access to a running wheel (22.5-cm diameter) attached to the side of the cage. Wheel revolutions were automatically registered with customized computer software. As reported previously, there was no effect on blood pressure, whereas body weights were increased in runners compared with controls at the end of the experiment period (13). Also, the running activity increased during the first 3 wk and thereafter plateaued and averaged at 16 km/24 h.

Ex Vivo Determination of Aortic Vessel Distensibility

Vessel preparation and ex vivo perfusion system. Ten additional SHR (5 runners and 5 controls) underwent an identical experiment...
protocol. At the end of the experiment period, the animals were decapitated and the descending thoracic aortae were immediately dissected out and placed in chilled physiological saline solution (0.9% NaCl) after measurement of the in vivo vessel length. The vessels were thereafter mounted in a specially designed vessel chamber, and the vessel segments were tied with two 3-0 silk laces to the cannulae in the perfusion line (Fig. 1A). A hydrostatic pressure device was used to create various intraluminal pressure levels, and the perfusion and incubation solutions were maintained at a constant temperature of 37°C. After 15 min of nonrecirculating equilibration under 60-mmHg perfusion pressure, the vessel segment was stretched to its in vivo length (~30%) and equilibrated for another 10 min. The flow was thereafter stopped, and the intraluminal pressure was increased from 50 to 150 mmHg with a 10-mmHg incremental step during simultaneous visualization of the vessel structure using an ultrasound biomicroscope system (UBM) (Vevo 600, Visualsonics, Toronto, Canada) equipped with a 55-MHz mechanical transducer, which provides a spatial resolution of 30 μm. Biological validation of this model, including vessel viability, has been described previously (11).

Data Analysis

Inner vessel lumen diameter was measured offline using the leading-to-leading edge principle from the near to the far wall intimal boundary (Fig. 1B). A pressure-cross-sectional area curve was thereafter created, and the paired data was also fitted to an arctangent men boundary (Fig. 1B). A pressure-cross-sectional area curve was obtained:

\[ \text{Compliance} (P) = \frac{\partial \text{CSA}}{\partial P} = \frac{\alpha}{\gamma} \frac{1}{1 + \left(\frac{P - \beta}{\gamma}\right)^2} \]

Finally, the pressure-distensibility curve was created by normalizing vascular compliance to CSA:

\[ \text{Distensibility} (P) = \frac{1}{\text{CSA}} \times \frac{\partial \text{CSA}}{\partial P} \]

Tissue Preparation

After the 5-wk training period, running wheels were locked 24 h before the day of death, when the 18 female animals were injected with an overdose of pentobarbital sodium. The aortae were dissected, divided, and treated according to subsequent experiment.

RNA extraction. RNA was extracted using Trizol reagent (Life Technologies, Invitrogen, Paisley, Scotland) according to the manufacturer’s protocol. In brief, aortae were pulverised using mortar and pestle, during cooling with liquid nitrogen, and homogenized with a power homogenizer. Fat and cell debris were removed by centrifugation (12,000 g, 10 min, 4°C). The RNA extraction was further obtained by addition of chloroform and isopropyl alcohol in subsequent steps. The RNA quality was verified spectrophotometrically using the SPECTRAmax Plus384 microplate reader (Molecular Devices, Sunnyvale, CA) and registered digitally by Soft Max PRO 3.1 (Molecular Devices). RNA purity (A260/A280) was set at >1.7.

Target Preparation and Gene Chip Hybridization

Double-stranded cDNA was prepared using Superscript choice system (Life Technologies, Paisley, UK) and an oligo(dT) 24-anchored T7 primer using 6.8 μg of aortic RNA from each rat. cDNA from runners (n = 9) and controls (n = 9) were pooled in groups of three. Biotin-labeled cRNA was synthesized from 10 μl of cDNA by in vitro transcription with biotin-labeled nucleotides and T7 RNA polymerase (Enzo Diagnostics, Farmingdale, NY). Labeled cRNA
was purified using RNeasy columns (Qiagen, Hilden, Germany), and 20 μg of cRNA were fragmented at 94°C for 35 min with 1× fragmentation buffer (40 mM Tris-acetate, pH 8.0, 100 mM KOAc, 30 mM Mg(OAc)₂) in a final volume of 40 μl. Fragmented cRNA was then used to prepare a hybridization mix with herring sperm DNA (0.1 mg/ml), and four control bacterial and phage cRNA (1.5 pM BioC, 25 pM BioD, and 100 pM Cre) samples to serve as internal controls for hybridization efficiency as directed by the manufacturer (Affymetrix, Santa Clara, CA). Aliquots of the hybridization cRNA mixtures (10 μg of cRNA in 200-μl hybridization mix) were hybridized to a RG-U34A DNA microarray, washed, stained, and scanned (Hewlett Packard, GeneArray scanner G2500A) according to procedures developed by the manufacturer (Affymetrix). Gene expression was analyzed on 3 × 2 DNA microarrays.

Microarray Gene Expression Analysis

To allow comparison of gene expression, the DNA microarrays were globally scaled to an average intensity of 100. Scanned output files were analyzed with Microarray Suite 5.0 software (Affymetrix). These files are available at the GEO database (http://www.ncbi.nlm.nih.gov/geo; series GSE2418).

RNA expression levels were estimated by two algorithms: 1) the signal algorithm, which computes the average difference between perfect-match and mismatched probe cells for each transcript, or 2) the detection call, which is given by an algorithm based on signal intensity and signal quality. With the detection call, a gene expression is classified as absent, marginal, or present (Affymetrix). For a gene to be classified as detectable and included in the analysis, it had to be called present in all three triplicates in either the running or control groups.

Genes with altered expression levels in running rats were identified by the change call algorithm, essentially as previously described (38). In brief, comparisons were made between the results from the triplicate DNA microarrays used for analysis of the running rats and the triplicate DNA microarrays used for analysis of the nonrunning rats, generating a total of nine comparisons. With the change call, a gene is classified as increased (I), marginally increased (MI), no change (NC), decreased (D), or marginally decreased (MD). A change call of D or MD was given a value of 1, a change call of MI or I was given the value of 1. A change call score was calculated by summarizing the values in all the nine comparisons for each probe set. The criterion for increased expression was a change call score of ≥7. The criterion for decreased expression was change call score equal to or less than −7. In addition, the regulated genes should have a P value of <0.05 between the signal values from the running vs. control groups (Mann-Whitney test). Genes were identified using the NetAffex database (www.netaffex.com).

Quantification of Gene Expression in Aortic Tissue

cDNA synthesis for real-time PCR. cDNA synthesis was carried out by reverse transcription (RT) using ThermoScript RT-PCR system (Invitrogen) according to the manufacturers’ protocol. In brief, 1 μg of total RNA was used in a total volume of 20 μl, containing cDNA synthesis buffer 1×: 5 mM DTT, 1 mM dNTP, 40 U Rnase OUT, 15 U Thermoscript RT. Samples were incubated at 25°C (10 min), 50°C (50 min), and 85°C (5 min) on a Corbett Thermocycler (Corbett Research, Sydney, Australia).

Relative quantification using real-time PCR. Relative quantification of mRNA expression was performed on a LightCycler (Roche Diagnostics, Mannheim, Germany). For amplification, 2 μl of diluted cDNA (1:8) were added to 18 μl of FastStart Master SYBR green I reaction mixture (Roche Diagnostics).

Oligonucleotides for LightCycler PCR assay. Oligonucleotide primers were designed using Primer Express version 1.0 (Perkin-Elmer Applied Biosystems) for GAPDH, and LightCycler Probe Design software version 1.0 (Roche Diagnostics) for HSP60 and HSP70, based on sequences from the GenBank database (Table 1). GAPDH was selected as endogenous control to correct for potential variation in RNA loading or efficiency of the amplification reaction.

Validation of PCR amplification. Standard curves for the three genes (GAPDH, HSP60, and HSP70) were obtained by plotting log dilution (y-axis) against crossing point (Cp) values (x-axis), as described previously (13). The correlation factor for linear regression analysis of the three studied genes was R² = 0.99, R² = 0.99, and R² = 0.97, respectively. Amplification efficiencies for the three genes, expressed as the slopes of the standard curves, were similar: −3.6, −3.7, and −3.2, respectively. The standard curves were subsequently used for calculations of the relative dilution value of each unknown sample.

Validation of endogenous control gene. The calculated relative dilution values for GAPDH, obtained by the second derivative maximum method (22), were similar in experimental and control groups (2.38 ± 0.34 and 2.48 ± 0.31; P = not significant).

Validation of specificity. Specificity of the PCR product is validated by melting-curve analyses. Furthermore, the final PCR product was verified as a single band on an agarose gel (data not shown).

Analysis of Protein Levels of HSP in Serum

Enzyme immunoassay (ELISA) was performed for HSP60 and HSP70 according to the manufacturer’s manual (HSP60 ELISA kit no. EKS-600 and HSP70 ELISA kit no. EKS-700, Stressgen Biotechnologies, Victoria, BC, Canada). The lower detection limits were 3.125 and 0.78 ng/ml for HSP60 and HSP70, respectively.

Immunohistochemistry

Thoracic aortic segments (3 mm) were fixed in 4% buffered formaldehyde, embedded in OCT compound (Sakura), and frozen in chilled isopentane. Sections of 6–10 μm were cut on a cryostat, and slides were stored at −20°C. After several standard pretreatment steps, sections were incubated with specific primary antibody (HSP60 or HSP70, no. SPA-806 and no. SPA-810, Stressgen; 1:100 dilution) for 24 h. Thereafter, slides were incubated with secondary biotinylated antibodies (dilution 1:400, no. BA-2001, Vector Laboratories) for 1 h and finally stained using Vectastain ABC and DAB kits (Vector Laboratories).

Statistics

Generally, PRISM 4.0 (GraphPad, San Diego, CA) was used for statistical analysis. Data are presented as means ± SE. Relative gene expression levels between runners and controls were compared using

Table 1. Oligonucleotide primers for real-time quantitative PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Oligonucleotide</th>
<th>Sequence</th>
<th>Accession Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH</td>
<td>Forward primer</td>
<td>5' - GAA CAT CAT CCC TGC ATC CA-3'</td>
<td>NM_017008</td>
</tr>
<tr>
<td></td>
<td>Reverse primer</td>
<td>5' - CCA GTG AGC TGC CGG TAC A-3'</td>
<td></td>
</tr>
<tr>
<td>HSP60</td>
<td>Forward primer</td>
<td>5' - GGC TAT CCC TAC TGC T-3'</td>
<td>X54793</td>
</tr>
<tr>
<td></td>
<td>Reverse primer</td>
<td>5' - GCA AGT CCC TGG TGC A-3'</td>
<td></td>
</tr>
<tr>
<td>HSP70</td>
<td>Forward primer</td>
<td>5' - TCA TCA AGA GCA ACT CG-3'</td>
<td>Z27118</td>
</tr>
<tr>
<td></td>
<td>Reverse primer</td>
<td>5' - CTT TGC GTG TGC ATG TC-3'</td>
<td></td>
</tr>
</tbody>
</table>

HSP, heat shock protein.
nonparametric Mann-Whitney test. α, β, and γ values were obtained using the nonlinear curving-fitting formula according to Langewouters et al. (18) in PRISM 4.0. The CSA- and distensibility-pressure relationships for runners and controls were finally analyzed using two-way repeated-measurement ANOVA. A P value of <0.05 was considered to be statistically significant. Once the overall ANOVA analyses were significant, post hoc testing was performed using Student’s t-test.

RESULTS

Aortic Vessel Distensibility

The CSA-pressure curve was shifted upward in runners compared with controls (2-way ANOVA, group × pressure, P < 0.0001) (Fig. 2A). Furthermore, the aortic vessel distensibility-pressure curve was shifted upward in running compared with control animals (2-way ANOVA, pressure × group, P < 0.0001) (Fig. 2B).

Expression Profiling of Rat Aorta

Gene expression in aortae from running and control SHR rats was analyzed on triplicate RG-U34A DNA microarrays (Affymetrix) analyzing ~7,000 full-length gene sequences and 1,000 expressed sequence tag (EST) clusters. In aortae from both running and control rats, ~3,000 genes were classified as detectable using the detection call algorithm. Regulated genes were identified as described in MATERIALS AND METHODS. Thirty-eight probe sets representing 18 identifiable nonredundant genes were classified as downregulated (Table 2). Fifty-three probe sets representing 38 identifiable nonredundant genes were classified as upregulated. The upregulated genes are under current investigation in our laboratory and are not reported in the present paper.

One striking pattern that was observed among the regulated genes was a downregulation of genes in the HSP family in runners (10 of 18 downregulated genes) compared with controls. The regulated HSP genes belonged to the small HSP, HSP40, HSP60, HSP70, HSP90, and HSP110 families (42). No HSP genes were classified as upregulated in the expression-profiling experiments. This indicates that aortae from running rats display a coordinated downregulation of HSP genes.

Heat shock transcription factors (HSFs) are the major transcription factors controlling the expression of HSP genes. The expression profiles were therefore researched to investigate whether transcriptional downregulation of HSF genes also occurred in aorta of running rats. Three probe sets for HSF1 were present on the DNA microarray. No difference was found between running and control groups. Neither was there any difference in gene expression of endothelial NO synthase between runners and controls.

Quantification of Gene Expression

HSP60 and HSP70 gene expression. A significant down-regulation was detected in HSP60 gene expression in runners compared with controls. Relative gene expression levels were 1.32 ± 0.13 in runners and 1.98 ± 0.16 in controls (P < 0.01) (Fig. 3). Like HSP60, gene expression of HSP70 was down-regulated by 83% in runners compared with controls after 5 wk of exercise training. Relative gene expression levels were 0.30 ± 0.15 in runners and 1.77 ± 0.45 in controls (P < 0.001) (Fig. 4).

Quantification of Protein Levels of HSPs in Serum

In the majority of the serum samples, HSP protein levels were undetectable. This was true for both runners and controls.

Immunohistochemical Protein Localization of HSP60 and HSP70

Immunohistochemical staining of HSP60 in aortic vascular sections revealed homogenous intimal and medial localization of HSP60 in both running and control rats. The HSP70 staining revealed a similar staining pattern as HSP60 (Fig. 5).

Tissue preparations incubated with media lacking primary antibody served as negative controls. No immunoreactivity was detected in these slides, which verified the specificity of the immunostaining.

DISCUSSION

In this study, we showed increased aortic vessel distensibility after voluntary exercise. We were also able to distinguish a coordinated pattern of gene expression downregulation of HSPs in response to voluntary physical exercise in SHR. The findings of downregulated HSP60 and HSP70 were further validated with real-time PCR, and their corresponding protein localizations in the aorta were demonstrated immunohistochemically.

At the level of transcription, the HSP response is regulated by the HSFs that interact with a specific regulatory element, the

Fig. 2. A: aortic pressure-cross-sectional area relationships in runners (■) and controls (□). Significant difference by 2-way ANOVA (P < 0.0001). B: aortic pressure-distensibility relationships in runners (■) and controls (□). Significant difference by 2-way ANOVA (P < 0.0001). *, **, and *** indicate significant levels of <0.05, <0.01, and <0.001 (post hoc t-test), respectively.
The heat shock element (HSE) present in the promoters of HSP genes. The HSFs are expressed constitutively and have to be activated and undergo trimerization to acquire their DNA-binding ability (33). This is in line with our microarray analysis indicating no gene regulation of HSF genes. Several mitogen-activated kinases (MAPKs) have been suggested to be important for the activation of HSFs in response to oxidative and mechanical stress in vascular cells (20).

Table 2. Genes classified as downregulated in rat aorta after exercise

<table>
<thead>
<tr>
<th>Affy ID</th>
<th>Gene Title</th>
<th>Gene Symbol</th>
<th>Score</th>
<th>C</th>
<th>R</th>
<th>Signal Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>rc_A1236601_at</td>
<td>Similar to HSP 105 kDa alpha</td>
<td>Hspb1</td>
<td>9</td>
<td>924</td>
<td>502</td>
<td>0.5</td>
</tr>
<tr>
<td>rc_A176658_s_at</td>
<td>Heat shock 27 kDa protein 1</td>
<td>Hspb1</td>
<td>9</td>
<td>3,985</td>
<td>2,614</td>
<td>0.7</td>
</tr>
<tr>
<td>rc_AA998683_g_at</td>
<td>Heat shock 27 kDa protein 1</td>
<td>Hspb1</td>
<td>9</td>
<td>2,651</td>
<td>2,437</td>
<td>0.7</td>
</tr>
<tr>
<td>M55534_s_at</td>
<td>Crystallin, alpha B</td>
<td>Cryab</td>
<td>9</td>
<td>4,915</td>
<td>3,165</td>
<td>0.8</td>
</tr>
<tr>
<td>rc_AA942685_at</td>
<td>Cytosolic cysteine dioxygenase 1</td>
<td>Cdo1</td>
<td>9</td>
<td>1,190</td>
<td>676</td>
<td>0.6</td>
</tr>
<tr>
<td>Z75029_s_at</td>
<td>Heat shock 70 kDa protein 1A</td>
<td>Hspa1a</td>
<td>9</td>
<td>1,312</td>
<td>852</td>
<td>0.6</td>
</tr>
<tr>
<td>L16764_s_at</td>
<td>Heat shock 70 kDa protein 1A/1B</td>
<td>Hspa1b</td>
<td>9</td>
<td>2,480</td>
<td>1,581</td>
<td>0.6</td>
</tr>
<tr>
<td>rc_AI007820_s_at</td>
<td>Heat shock 90 kDa protein 1, beta</td>
<td>Hspcb</td>
<td>9</td>
<td>1,707</td>
<td>1,126</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Genes that are missing titles or symbols were not defined in netaffx. C, controls; R, runners.

heat shock element (HSE) present in the promoters of HSP genes. The HSFs are expressed constitutively and have to be activated and undergo trimerization to acquire their DNA-binding ability (33). This is in line with our microarray analysis indicating no gene regulation of HSF genes. Several mitogen-activated kinases (MAPKs) have been suggested to be important for the activation of HSFs in response to oxidative and mechanical stress in vascular cells (20).
HSP60 shows a highly homologous sequence between different species, from bacteria to humans. Both chlamydial and human HSP60 have been shown to have cytokine-like activity and induce tumour necrosis factor-α and matrix metalloproteinase production in human and mouse macrophages (16). HSP60 binds to endothelial cells and macrophages via the Toll-like receptor 4/CD14/MD2 receptor complex (23, 35), leading to activation of a signaling cascade, eventually activating nuclear factor-κB and synthesis of proinflammatory mediators, e.g., E-selectin, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1. Furthermore, antibodies against HSPs have been suggested to exert direct cytotoxic effects through complement activation (34).

Although HSP60 is considered to be mostly expressed in mitochondria in response to various types of stresses, it has also been found on the surface of stressed endothelial cells and macrophages (37, 44). Extracellular HSP60 and HSP70 have been shown to induce production and release of proinflammatory cytokines (1, 16). HSPs can be released to the extracellular space during cell death or cell injury. However, spontaneous release of HSPs via mechanisms suggested to involve lipid rafts have been described (3). Also, Lancaster and Febbraio (17) very recently reported a novel release mechanism for HSP70 involving membrane vesicles (exosomes). Furthermore, Xu et al. (43) found that HSP60 also exists in a soluble form in the circulation, which is positively correlated with the severity of atherosclerosis, suggesting a triggering role for HSP60 in an arterial inflammatory reaction. Also, serum antibodies to the mycobacterial HSP65 (mHSP65) have very recently been reported to be associated with elevated coronary calcification (46). Studies in mice show that vaccination with mHSP65 reduces the inflammatory process associated with atherosclerosis (21). Although there could be distinct mechanisms controlling transcription, translation, and release of this chaperone, it is still conceivable that the observed coordinated downregulation of HSP gene expressions in the aortae of running rats may lead to a reduction of proinflammatory cytokine release in the aorta. This may represent a novel anti-atherogenic mechanism of voluntary exercise.

The role of HSP70 in the development of atherosclerosis is somewhat disputed in the literature. High serum levels of the inducible form of HSP70 seem to be of protective role against atherogenesis (47). Gene transfection of HSP70 provides cardioprotection against ischemia-reperfusion injury (14), and immunization against HSP70 facilitates intimal thickening in balloon-injured rats (12). However, lower anti-HSP70 antibody levels have been observed in patients with intermittent claudication, critical lower limb ischaemia, and aneurysms (6, 27).

After acute physical exercise, HSP70 mRNA levels have been shown to increase transiently in rats, as well as HSP70-positive leukocytes in athletes (10, 32). Also, mRNA expression of HSP72, the inducible form of HSP70, in skeletal muscle was increased during acute cycling (9). In the present study, to avoid acute effects and study long-term effects of exercise, running wheels were locked 24 h before death. As evident from the study by Salo et al. (32), the acutely increased HSP70 levels in skeleton muscles already return to baseline after 6 h. Similar observations were made by Ferenbach et al. (10), who found that the acutely increased levels of HSP60 and HSP70 in human leukocytes were normalized 24 h postexercise. Indeed, at rest, HSP70 expression in leukocytes was significantly lower in endurance athletes compared with untrained individuals. This has been suggested as a consequence of adaptation upstream of the HSP level, such as increased antioxidative capacity. This may support our theory of increased vascular NO bioavailability after chronic voluntary exercise (13).
Regulation of HSP expression has been shown to occur mainly at the level of transcription (26), and intracellular overexpression of HSPs has been shown to lead to translational of the proteins to the cell surface. Using immunohistochemistry, we showed that HSP60 and HSP70 proteins are localized throughout the vessel wall with predominant intracellular distribution. Also, by assessing serum levels of HSP60 and HSP70, we failed to show any detectable levels of these proteins in SHR serum. Indeed, many posttranscriptional and translational processes could be involved to determine the final functionality of HSP. Also, the potential time lag between the transcriptional and translational steps may also contribute to the lack of apparent difference between runners and controls regarding the release and vascular expression of HSP proteins in the present study.

It is conceivable that, in these relatively healthy animals without severe atherosclerotic lesions, the HSP release into the circulation is still low. Furthermore, the lack of detectable systemic levels in SHR cannot exclude local release or effects of HSPs in the vascular wall. The source and regulatory mechanisms underlying the soluble HSPs in serum in man is still debated. Regarding HSP60, increased levels have been associated with more advanced vascular disease stages (43). Further studies are indeed required to address this interesting and complicated issue.

Given that the heat shock factor is an acute stress transcription factor, mediating the de novo synthesis of HSP, the concerted downregulation of HSP family members may indicate decreased vascular stressor stimuli. It is likely that the decreased HSP gene expression is a consequence of physical activity rather than a cause to the improved vascular function following exercise, at least in this relatively healthy animal model. However, this HSP regulatory mechanism could be of potential importance in, for example, animal models of atherosclerosis with advanced vascular lesions. Indeed, physical exercise has been shown to have beneficial effects on both progression and regression of atherosclerosis in mice (30, 31). However, potential roles of HSP have, to the best of our knowledge, not been explored in these exercised animals with human disease. Thus the gene regulation data from the microarray analysis may provide indexes of early involvement of HSPs in exercise-induced vascular effects. Future studies of the time windows of the transcriptional and translational regulations of HSP, as well as usage of, for example, exercising atherosclerotic mouse models, may shed further light on potential roles of the genetic regulation of HSPs for development of atherosclerotic lesions.

High-resolution UBM has been used successfully to image mouse cardiovascular morphology and function in vivo (45). Using a custom-designed small-vessel perfusion chamber, morphology of the isolated aortae could be clearly visualized using UBM. To extend the previous in vivo observation of increased aortic compliance (13), the isolated vessels were studied in a physiological saline solution instead of calcium-free solution, used when only the passive vascular mechanical properties are studied. Apparently, this in vivo approach facilitates extended physiological studies of the vessels also at the parophysiological pressure levels. Furthermore, thanks to the accurate measurement of the vessel lumen diameter, potential confounding effects of different vessel calibers can be taken into account.

Vascular distensibility is controlled by both structural and functional components in the vascular wall (39). By mechanically removing the endothelial cells, Boutouyrie et al. (2) demonstrated a decrease in aortic compliance. This may indicate a pivotal role for endothelial-derived NO in the acquisition of high vascular compliance. Moreover, exercise-induced flow increase has been shown to initiate a vascular remodelling process, resulting in enlarged arteries (8, 25). The adaptation is suggested to be a consequence of increased shear stress levels (40), which trigger NO-dependent vasodilation to normalize wall shear (39).

Exercise has been shown to generate production of ROS in the vasculature, which is believed to be a consequence of increased systemic metabolism. Simultaneously, there is also increasing evidence that exercise attenuates oxidative stress by upregulating the defense system against free radicals (15). The elevation of ROS levels has several consequences in the vascular wall, e.g., inactivation of NO, lipid peroxidation, and triggering of stress protein synthesis (19, 29). Indeed, in a previously published study (13), our laboratory showed a significant upregulation of CuZnSOD in aortic tissues from the exercised animals compared with their sedentary counterparts. Also, independent of vessel caliber, exercise improved aortic wall distensibility, which could be a consequence of increased vascular NO availability. Furthermore, in the same study, our laboratory performed functional evaluations of the influence of free radicals on acetylcholine-induced vasodilation in resistance arteries ex vivo. When vessel segments were incubated with a free radical scavenger (MnTBA), the increased sensitivity of acetylcholine in exercised animals compared with controls was abolished, indicating higher levels of oxidative stress/low antioxidant capacity in sedentary animals. This data is in line with findings by other groups and indicate an increased antioxidative capacity in vascular tissue in response to chronic exercise. Thus the coordinated downregulation of the de novo synthesis of HSP family members could be a consequence of decreased ROS production due to an increased vascular antioxidative capacity in the exercised animals.

In conclusion, voluntary physical exercise induced concerted downregulation of HSP gene expressions with concomitant improvement of aortic wall distensibility. Potential causal relationships between HSP expression and vascular function may justify further investigations.

ACKNOWLEDGMENTS

We thank laboratory technicians Gunnel Andersson and Jing Jia for excellent technical assistance.

GRANTS

This work was supported by grants from the Swedish Medical Research Council (project LG 14601, 14602, and 15168), the Swedish Heart-Lung foundation, the Åke Wiberg Foundation, Swegene, the Memorial Foundation of Lars Hierra, and the Magnus Bergvall Foundation.

REFERENCES

EXERCISE, HSP GENE EXPRESSION, AND AORTIC DISTENSIBILITY


